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Red jasmine rice bran extract for the prevention of the blue light induced photodamges

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Abstract: *Blue light protection is one of the hottest topics in sun care categories recently due to blue light would result in skin photodamages such as oxidative stress, pigmentation and photoaging. In this research, a blue light induced cellular injury model was constructed and the blue light exposure could indeed lead to ROS accumulation in HaCat cells, melanin secretion enhancement in the B16 cells and COL1A1 gene expression reduction in the HFF cells. Red jesmine rice bran extract was previously studied to have good UV protection benefit. In this study its blue light protection benefits were further investigated. Red jesmine rice bran extract can prevent the cellular damages caused by the blue light based on the blue light induced cellular injury model including preventing the ROS accumulation in HaCat cells, inhibiting the melanin secretion in the B16 cells and enhancing the COL1A1 gene expression in the HFF cells. Red jesmine rice bran extract could be potentially applied in sun care products to prevent the blue light induced photodamages.*

Keywords: *sun care, blue light, blue light protection, red jesmine rice bran extract*

1. Introduction

Solar radiation refers to the continuous electromagnetic radiation that reaches the earth, which can be divided into infrared light, visible light and ultraviolet light. Among them, blue light is the band with the shortest wavelength and the highest energy in the visible spectrum. Studies have shown that the abundance of blue light is more than twice that of UVA in ultraviolet[1](including UVA (90% -95%) ,UVB (5%-10%), UVC), its penetration is stronger than ultraviolet light and 20% of it can even reach the hypodermis[2].Blue light is able to cause similar damages to human skin as the ultrovoliet light, such as oxidative stress[3], persistent pigmentation[4] and extracellular matrix (collagen) degradation[5,6].

In sun care application, there are basically four photoprotection pathways that could be applied to protect photodamages including UV and HEV specific filtering, ROS synthesis inhibition, melanin secretion inhibition and collagen degradation inhibition. Studies have shown that sunscreens containing only phyiscal and chemical UV filters are not enough to protect the ROS generated from the sunlight, especially from the HEV[3,7]. Biological sun protection ingredients from a natural source of plants or microorganisms have sunlight protective effect. The main mechanism of action listed is sunlight absorption, anti-inflammation [8] and anti-oxidative stress [9], which is a supplement to physical and chemical sunscreen filters that can protect both UV and HEV.

In the previous work, lots of effort for biological sunscreen filters have been focused on SPF, UVAPF boosting effect in terms of UV protection[9-14]. In 2012, Frank Liebel et al.[3] have found that a sunscreen with the biological antioxidants composition composing of Feverfew (T. parthenium) extract, Soy (G. soja) extract, and Gamma Tocopherol was able to protect the damages induced by blue light including ROS generation, inflammation and MMP-1 secretion. Until recently blue light protection becomes a trendy research and more and more commercial biological ingredients have been developed with the purpose of blue light protection to prevent photodamages, for example Lumicease™ from Lubrizol, Cite'M™ from BASF and Blumilight™ from Ashland etc. The majority of these biological ingredients has anti-oxidation effect which is able to protect the negative biological impacts induced by the blue light[15].

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Rice is a food consumed by people all over the world. It is a grain harvested from a plant called Oryza sativa. The whole grain is composed of the inedible hull and the edible layers inside which are bran, kernel and endosperm, respectively. In Asian, rice is the main crop and has quite a big number of cultivated varieties which include the variety with different colors. Yakaew et al. has previously found out that hydroglycolic crude extract of red jesmine rice bran contains procyanidinin which showed comparable DPPH radical scavenge property to Vitamin C. Meanwhile it can also prevent UV induced MMP-1 secretion whiling promoting the extracellular type I procollagen[16]. Due to red jesmine rice bran extract show pronounced anti-oxidation property and UV protection benefit, we would like to further investigate its potent in blue light protection properties.

To understand how blue light induced skin damages and whether the red jesmine rice bran extract could prevent these damages, we have constructed a blue light induced skin damages cellular injury models starting from the outside layer (the keratinocytes), to the middle layer (the melanocytes), and finally to the deeper layer (the fibroblasts) and then apply the red jesmine rice bran extract to this model. We have found out that the blue light could induce ROS accumulation in the keratinocytes, melanin secretion in the melanocytes, and COL1A1 gene expression reduction in the fibroblasts. While applying the red jesmine rice bran extract could reverse these trends by decreasing the ROS generation in the keratinocytes, inhibiting melanin secretion in the melanocytes and boosting the COL1A1 gene expression in the fibroblasts. Red jesmine rice bran extract could be potentially applied in sun care products to prevent blue light induced skin photodamages.

2. Material and methods

2.1 Materials

Red jesmine rice bran extract was prepared based on the methods published previously[16].Briefly speaking, the rice bran was immersed in a 50% hydroglycol solution at a ratio of 1:7 for three days. The macerate was filtered, leaving the filtrate to be used as crude hydroglycolic extract of the rice bran.

2.2 Methods

2.2.1 ROS level test induced by blue light

The blue light induced injury model was constructed by irradiating HaCaT, HFF and B16 cells with blue light. After adding the red jesmine rice bran extract (0.025% as the raw material), the cell culture was cultured for 24h. Then the antioxidant capacity of test groups was determined by detecting the level of active oxygen species in cells. The specific methods are as follows:

1) Take the cells in good condition in the logarithm growth period and digest them with trypsin. Then resuspend the cells and inoculate them into 96 well plates at the amount of 10000 cells/well. Continue to culture the cells until 80% of the fusion is started. The cells were divided into blank control group, blue light irradiation positive control group and the red jesmine rice bran extract treated group upon blue light irradiation. The irradiation condition was 18 MW/cm^2 for 1 h. Then the cell culture was cultivated for additional 24 hour.

2) The cells were digested and collected. They were resuspended in PBS solution, and the cell precipitate was collected. Dilute DCFH-DA with serum-free medium at 1:1000 to a final concentration of 10μM. Resuspend the collected cell pellet in diluted DCFH-DA medium, and place it in a cell incubator at 37℃ for 20 minutes. Mix it upside down every 3-5 minutes to allow full contact between the probe and the cells. After the incubation was completed, the cells were washed three times with serum-free culture medium to fully wash away DCFH-DA that was not bound to the cells. The final cell pellet was resuspended in PBS and added to the fluorescent detection plate(96-well full blackboard)in an amount of 100 μL per well. Set up three duplicates for each sample, and the remaining part of the cell suspension was used for cell number correction.

3) Put the fluorescence detection plate into a multi-functional microplate reader to detect the florescence according to the DCFH probe detection principle. Calibrate the cell number to obtain the relative fluorescence intensity of each sample.

2.2.2 Melanin inhibition test induced by blue light

The cells were cultured and processed according to steps 2.2.1. The supernatant was discarded and washed with PBS three times. Then 200 μL of 1 mol/L NaOH solution(containing 10% DMSO)was

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added to each well. The cells were fully lysed at 80℃ for 1 hour, and the absorbance of each well was measured at the wavelength of 475 nm. The protein content in the solution was determined using the BCA method.

2.2.3 Collagen gene expression induced by blue light

The Cells were cultured and processed according to steps 2.2.1. Then the cells were washed and 1ml of TRIZOL was added to lyse the cells. The total RNA of the cells was extracted using the classic chloroform and isopropanol methods, and mRNA was reverse transcribed using a reverse transcription kit to synthesize cDNA. The SYBR probe method was then used to quantitatively detect COL1A1 genes.

2.2.4 Statistical analysis

Statistical analysis was done using GraphPad Prism 7.0 software (GraphPad Software Inc., San Diego, CA, USA). The data were displayed as mean±SD values. Statistical comparisons between different treatments were made using two-tail t-test. Statistical significance was set at $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

2.3 Results

In the blue light induced cellular injury ROS model in the HaCat cells, the ROS production was significantly enhanced which proved that the model system was successfully constructed. When treated with the red jesmine rice bran extract together with exposure to the blue light, the ROS production show significantly reduced which proved that the red jesmine rice bran extract could prevent ROS accumulation after blue light irradiation. (Figure 1) In this set of study, the red jesmine rice bran extract could prevent the blue light induced ROS accumulation in the keratinocytes.

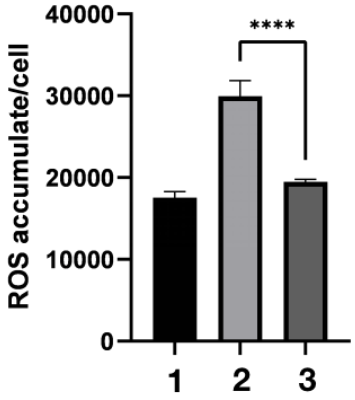

Figure 1: ROS accumulation with 1) the control group; 2) blue light irradiation without product treatment group; 3) blue light irradiation with the treatment of red jesmine rice bran extract group.

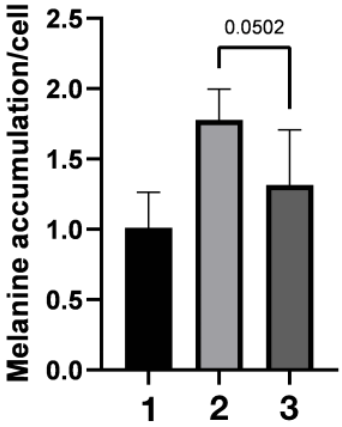

Figure 2: Melanin secretion with 1) the control group; 2) blue light irradiation without product treatment group; 3) blue light irradiation with the treatment of red jesmine rice bran extract group.

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In the blue light induced cellular injury melanin secretion model in the B16 cell, the melanin secretion was significantly enhanced which proved that the model system was successfully constructed. When treated with the red jesmine rice bran extract together with exposure to the blue light, the melanin production was decreased. Although there were no significantly difference between the product treated and untreated group, the P value is close to 0.05 and the decreasing trend of melanin production after red jesmine rice bran extract treatment is obvious.(Figure 2) In this set of study, the red jesmine rice bran extract can inhibit the melanin production caused by the blue light which show its potent to prevent the blue light induced skin pigmentation.

In the blue light induced cellular injury collagen synthesis model in the HFF cell, the production of COL1A1 gene was significantly decreased which proved that the model system was successfully constructed. When treated with the red jesmine rice bran extract together with exposure to the blue light, the gene expression of COL1A1 was significantly enhanced which proved that the red jesmine rice bran extract could prevent the COL1A1 degradation induced by blue light. (Figure 3) In this set of study, the red jesmine rice bran extract showed its potent to prevent the blue light induced photoaging problem.

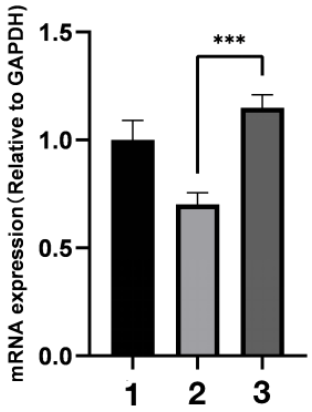

Figure 3: COL1A1 gene expression with 1) the control group; 2) blue light irradiation without product treatment group; 3) blue light irradiation with the treatment of red jesmine rice bran extract group.

2.4 Discussions

As recognized by researchers, 80% of facial aging is derived from sunlight exposure[17]. Blue light, serve as one of the important components from sunlight, can penetrate deeper to the skin comparing with UV light[2]. Continuously exposure to blue light can lead to skin pigmentation, darkness, unevenness of color distribution[18]and wrinkles[19]. Thus, we have constructed a blue light induced skin damages cellular injury models starting from the outside layer (the keratinocytes), to the middle layer (the melanocytes), and finally to the deeper layer (the fibroblasts) . Our results shows that this blue light induced cellular injury model was successfully constructed by increasing the ROS accumulation in the HaCat cells, promoting the melanin production in the B16 cells and decreasing the COL1A1 gene expression in the HFF cells.

On the other hand, red jesmine rice bran extract is an effective blue light protection agent which is able to prevent the photodamages induced by blue light based on our cellular study. By applying red jesmine rice bran extract, the celluar damages induced by blue light can be reversed including decreasing the ROS generation in the HaCat cells, inhibiting the melanin production in the B16 cells and enhancing the COL1A1 gene expression in the HFF cells. Based on these set of study, the red jesmine rice bran extract could be potentially added to the sun care product to prevent the blue light induced photodamages such as oxidative stress, pigmentation as well as photoaging.

3. Conclusions

In this research, we have successfully constructed a blue light induced cellular injury model including ROS generation enhancement, melanin secretion enhancement and COL1A1 gene expression reduction in HaCat, B16, HFF cells respectively. Meanwhile, we have also proved that the application of red jesmine rice bran extract could protect the cellular damages which could be potentially prevent the ROS accumulation, pigmentation and photoaging problems caused by the blue light.

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